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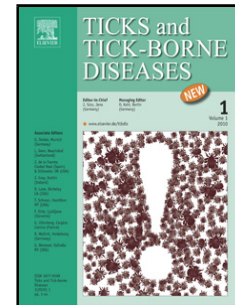
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**"Meeting the challenge of tick-borne disease control: A proposal for 1000 *Ixodes* genomes"**

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## Introduction

At the ‘One Health’ 9th Tick and Tick-borne Pathogen Conference and 1<sup>st</sup> Asia Pacific Rickettsia Conference (TTP9-APRC1; <http://www.ttp9-aprc1.com>), 27 August-1 September 2017 in Cairns, Australia, members of the tick and tick-borne disease (TBD) research communities assembled to discuss a high priority research agenda. Diseases transmitted by hard ticks (subphylum Chelicerata; subclass Acari; family Ixodidae) have substantial impacts on public health and are on the rise globally due to human population growth and change in geographic ranges of tick vectors (de la Fuente et al., 2016). The genus *Ixodes* is a global menace. Members of the genus impact human and animal health directly via host parasitism, and indirectly via transmission of multiple viral, bacterial and protozoan diseases. The first tick genome assembly was completed in 2016 for *Ixodes scapularis* (blacklegged tick), the North American vector of Lyme disease (LD), human babesiosis, human anaplasmosis and Powassan virus (Gulia-Nuss et al., 2016). The assembly provided insight into the genome biology of hard (ixodid) ticks and supported molecular studies for many species of Acari (ticks and mites). Draft genome assemblies are available for *Ixodes ricinus* (castor bean tick; Cramaro et al., 2015; 2017) and *Rhipicephalus (Boophilus) microplus* (southern cattle tick; Barrero et al., 2017). However, high quality reference assemblies to rival those produced for the mosquito vectors *Anopheles gambiae* (Neafsey et al., 2015) and *Aedes aegypti* (Mathews et al., 2017), have not been produced for a tick vector. Conference attendees identified the need to expand genomic resources for tick research, beginning with the genus *Ixodes* – one of the most important phyletic groups affecting human and animal health worldwide. Inspired by the 1000 *Anopheles* genomes effort (Malaria Genomic Epidemiology Network, Ag1000G), an ambitious goal to sequence and assemble the genomes of 1000 *Ixodes* ticks was proposed. The *Ixodes* 1000 genomes project (Ix1000G) outlines a “hub and spoke” model to sequence both laboratory reference strains and natural populations of *Ixodes*. The project is aligned with other ambitious genome initiatives such as the i5K effort that proposes to sequence 5000 arthropod species (Evans et al., 2013) and the Earth BioGenomes project, a moonshot to sequence and catalog all of Earth’s biodiversity (Lewin et al., 2018). The *Ixodes* 1000 Genomes Consortium (IGC) represents an international scientific collaboration formed to launch and guide the initiative. This Letter to the Editor defines the strategic vision of the Ix1000G and serves as a call to the broader scientific community for engagement.

## The need for tick genome resources

Tick-borne diseases affect human and animal health globally. Lyme disease (LD) is one of the most important TBDs in North America and Europe. It is estimated that the approximately 30,000 human cases reported to the U.S. Centers for Disease Control and Prevention (CDC) each year represent only about 10% of actual cases (CDC; Hinckley et al., 2014; Nelson et al., 2015). In Europe, roughly 85,000 LD cases are reported annually, although actual case numbers are unknown (European Centre for Disease Prevention and Control (ECDC), 2012). Recent studies are also shedding light on the transmission of human and animal pathogens by Australian ticks and the role of *Ixodes holocyclus*, as a vector (reviewed in Graves and Stenos, 2017; Greay et al., 2018). Options to control hard ticks and the pathogens they transmit are limited. Human vaccines are not available, except against the tick-borne encephalitis virus (Heinz and Stiasny, 2017). Diagnosis and treatment of many TBDs is a challenge because reliable, comprehensive molecular and immunological diagnostics are lacking. Control is also complicated by environmental impacts of acaricides and development of resistant tick populations. Human-tick encounters and pathogen transmission are increasing as geographic ranges of ticks expand and humans exploit new habitats. The need for new strategies to control ticks has never been greater. This need is reflected in recent funding announcements from the U.S. National Institutes of Health (NIH), the Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP), FY17 “Tick-Borne Disease Research Program (TBDRP)”, and the U.K. Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC) “Networks in Vector Borne Disease Research”. Pressing questions remain in TBD research: how do pathogen complements vary in tick populations at local and regional scales? How do tick microbiomes (including symbionts) and vector genetics affect pathogen transmission and disease outcome? How can scientists best develop comprehensive, region-specific diagnostics, risk matrices and treatment regimes? Fortunately, advances in sequencing and the rapidly-evolving field of data science offer the tools to address many of these questions. The *I. scapularis* genome sequence (Gulia-Nuss et al., 2016) and other molecular and genetic resources for a number of *Ixodes* species such as transcriptome, sialome and mialome datasets (reviewed in Chmelař et al., 2016; Rodriguez Valle et al., 2018), and the establishment of the DNA and tissue repository via the Global Genome Biodiversity Network (Gonzales et al., 2017), are an important beginning. However, incremental advances in science will not be adequate to meet pressing public health needs; ambitious goals must be proposed to effect a rapid paradigm shift in TBD research.

## **Data-driven solutions for tick and tick-borne disease control**

Multiple species of ticks and mites of medical significance have been recognized as high priority targets for sequencing by the NIH (Hill, 2010; Gulia-Nuss et al., 2016). These projects were not advanced due to the limitations of sequencing platforms available at the time. Recently, ultra-long read, single-molecule and genome scaffolding technologies have enabled production of genome assemblies for multiple eukaryotes, including mosquitoes (Jiao et al., 2017; Zimin et al., 2017; Ag1000G, 2017; Mathews et al., 2017). The genomes of 765 field-collected *Anopheles gambiae* and *Anopheles coluzzii* from 15 locations were sequenced by the Ag1000G project (Ag1000G, 2017). The *Ae. aegypti* L5 genome assembly produced by Matthews et al. (2017) comprises chromosome-length scaffolds produced via a hybrid PacBio (Pacific Biosciences) and HiC (Dovetail Genomics) approach. These landmark achievements suggest that modern sequencing technologies will have utility for assembling the large, repeat-rich genomes of ixodid ticks.

The broad objectives of the *Ix*1000G are to: (1) build multiple reference assemblies to support research on *Ixodes* vectors in North America, Europe, Africa and the Asia-Pacific, (2) catalogue natural genetic variation among wild populations of *Ixodes* and their microbial assemblies, (3) describe the genetic history and population structure of *Ixodes* species, and (4) combine genetic variation with biological data to understand tick ecology, epidemiology and disease transmission. The *Ix*1000G will generate fundamental resources for identification and analysis of tick genes and other genomic features, and will support three critical areas needed to advance research on tick and TBD control:

*The systems biology of tick-host-pathogen interactions.* Every tick bite represents a unique, complex molecular interaction between the tick, the pathogen and the immunological landscape of the vertebrate host. Potentially hundreds of tick salivary factors and microbes are delivered to the host dermis during blood feeding, which lasts for days to over a week. Furthermore, molecular interactions between ticks and commensal or pathogenic microorganisms within tick microbiota may also shape pathogen infection and transmission. Genomics offers opportunities to unravel the systems biology of tick-host-pathogen interactions across multiple *Ixodes* species.

*The link between tick genetics, vector competence and disease transmission.* To understand this link, there is need to evaluate the correlation between the genetic structure of tick populations and their microbial complement, including pathogens and symbionts. Molecular studies have revealed multiple genetic clades, signatures of north-south population structure (Gulia-Nuss et al., 2016), and evidence for gene flow between populations of *I. scapularis* (Van Zee et al., 2015), but implications for pathogen transmission remain unclear. As sequences of tick-associated microbes are typically recovered with tick genome data, results from the *Ix*1000G will provide fundamental insight into this tripartite relationship.

*Genetic frameworks for *Ixodes* control.* Population genomic studies can directly inform pest management strategies for *Ixodes* and other tick pests. Applications may involve the development of acaricides with species-selective activity and innovative approaches through microbiome manipulation to disrupt pathogen transmission similar to strategies being explored for triatomine bugs and tsetse flies (Hurwitz et al., 2012; Medlock et al., 2013). Population genetics data can also support the development of resistance management and genetic control strategies. For example, genome-wide analyses revealed high levels of heterogeneity between hundreds of *Anopheles* in Africa, suggesting potential for introduction of resistance alleles and evolution of insecticide resistance (Ag1000G, 2017). This indicates the need for genetic control programs tailored to the genetic background of local mosquito populations, and molecular analyses of resistance alleles suggest the same phenomenon will apply to tick populations.

### **The *Ix*1000G roadmap**

The *Ix*1000G strategy calls for whole genome deep sequencing to generate reference assemblies for multiple *Ixodes* species (Priority Area 1) as well as a high-resolution view of genetic variation and microbiome structure in natural populations across the genus (Priority Area 2). Global project partners will source materials and work with teams skilled in data analysis to execute the project in phases, with oversight provided by the IGC. Importantly, the IGC will define a set of standards for genome assembly and annotation aligned with those recognized by sequencing institutes and the Genomic Standards Consortium (Chain et al., 2009; <http://gensc.org/>).

#### **Priority area 1: High quality reference assemblies for *Ixodes* node species**

The Consortium proposes sequencing and assembly of up to six node species via a combination of PacBio ultra-long-read technology and Hi-C scaffolding of assembled regions. This approach has been used to

generate high quality reference assemblies for *An. gambiae* and *Ae. aegypti* (Ag1000G, 2017; Matthews et al., 2017). The *Ixodes* species listed in Figure 1 have been identified as high priority sequencing targets on the basis of medical significance and potential to anchor additional genome studies across the genus *Ixodes*. This includes population genomic studies as described under Priority Area 2 below. Sequencing will generate a high-quality reference assembly for *I. scapularis*, building on the existing draft genome. Efforts to generate an improved assembly for *I. ricinus* are already underway via the GenIric project (<http://www.angers-nantes.inra.fr/en/All-the-news/GenIric>). Reference genomes will support production of assemblies for other species of *Ixodes*, as well as alignment and analysis of genomic data from field-caught specimens. Species such as *Ixodes affinis*, *Ixodes uriae* and *Ixodes cookei* that are thought to play a minor role in disease transmission, or that do not primarily parasitize humans, are recommended for comparative analyses. The engagement of laboratories with the capability to source ticks and prepare genetic material for sequencing will be critical to these efforts (see Table 1). To obtain sufficient DNA for long read libraries (PacBio, 10X or Nanopore) and reduce heterogeneity in sequenced samples, the IGC proposes to sequence one individual of each node species (or a minimal number). In-bred reference strains should be established via sib-matings for each node species in the case of species listed in Table 1 that have not already been colonized. This would provide source material of similar genetic background for Chicago and Hi-C libraries to support genome scaffolding, and complementary transcriptomic and proteomic studies to validate predicted coding sequences and analyses of gene expression.

The IGC also identified the generation of reference assemblies for tick cell lines in regular use by the scientific community as a priority (Oliver et al., 2015; Miller et al., 2018; Tick Cell Biobank: <https://www.liverpool.ac.uk/infection-and-global-health/research/tick-cell-biobank/>; Table 1). Forward genetic approaches can be used to identify genes associated with vector competence and acaricide resistance. Inbred strains of ticks with quantifiable traits will facilitate genome-wide association studies (GWAS) and mapping of quantitative trait loci (QTL) that must be developed.

#### *Priority area 2: Genomics of natural Ixodes populations and microbiome composition/structure*

Under this priority area, the Consortium recommends deep sequencing of several hundred individual ticks belonging to the six node species via an Illumina approach. These should be collected from 10 or more geographic locations across North America, Europe, Africa and Asia. Dual digest Restriction Site Associated DNA sequencing (ddRADseq) may offer a complementary and more financially-viable strategy to generate population genomic data. These studies will also reveal sequences of symbionts and pathogens, and provide insight into the microbiome structure of *Ixodes* at macro-geographic scales. The initial Ix1000G goal proposes a target of 1000 genomes, but the effort is expected to encourage generation of genome data from many thousands of individual ticks in subsequent phases.

#### **Conclusions and Broader Impacts**

The Ix1000G will provide a critical framework to support genome studies of other ticks, and mites, of medical importance, including members of the metastriate genera *Amblyomma*, *Rhipicephalus* and *Dermacentor* that include multiple vector species. Moreover, genomes for several mite species of medical importance are already available (Chan et al, 2015; Mofiz et al., 2016; Rider et al., 2017), including a recent genomic analysis of the scrub typhus vector *Leptotrombidium deliense* (Dong et al., 2018). These members of the Acari, with smaller nuclear genomes compared to hard tick species, will support genomic analyses while the more challenging reference assemblies and population genomic studies of wild *Ixodes* populations are in development. Given the worldwide medical and veterinary importance and smaller nuclear genome size of *Ixodes* species as compared to metastriate ticks, coupled with the availability of supporting genomic resources, the genus *Ixodes* represents a logical starting point for generation of high quality reference assemblies and population genomic data. This Letter to the Editor is an official call for project partners and strategic investment to advance the Ix1000G initiative. The broader, long-term vision is an assemblage of data from many thousands of *Ixodes* ticks and a detailed understanding of genetic diversity among *Ixodes* populations worldwide. Data will be used to understand the transmission of TBDs in the context of tick genetics and microbiome communities. The Ix1000G will operate across geographic boundaries to provide the first global genomic resource for tick research and help meet the Grand Challenge of tick and TBD control on a global scale.

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ACCEPTED MANUSCRIPT

**Figure Legends:**

**Figure 1. Schematic showing six “node” species of *Ixodes* recommended as targets for deep genome sequencing.** The species *Ixodes scapularis*, *Ixodes pacificus*, *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes rubicundus* and *Ixodes holocyclus* were selected based on status as disease vectors and their potential to anchor population genomic studies across the genus *Ixodes*. Segments of the pie chart show diseases transmitted or caused by ticks: BAC, Bacterial Artificial Chromosome library; LD, Lyme disease; HGA, Human granulocytic anaplasmosis; TBE, Tick-borne encephalitis; SFGR, Spotted fever group rickettsiosis.

**Table 1. *Ixodes* species proposed for genome sequencing (priority area 1) and availability of colonies and cell lines for sequencing.**

Tick Species		Pathogen Transmitted	Disease	Genome Project Resources
Scientific Name	Common Name			
North America				
<i>Ixodes scapularis</i>	Blacklegged tick	<i>Borrelia burgdorferi</i> <i>Babesia microti</i> <i>Anaplasma phagocytophilum</i> Powassan virus <i>Borrelia miyamotoi</i>	LD Babesiosis HGA Powassan encephalitis <i>Borrelia miyamotoi</i> disease	Colonies; UTMB; BEI Resources <sup>a</sup> ; field specimens
<i>Ixodes pacificus</i>	Western blacklegged tick	<i>Borrelia burgdorferi</i> <i>Anaplasma phagocytophilum</i> <i>Borrelia miyamotoi</i>	LD HGA <i>Borrelia miyamotoi</i> disease	Colonies; BEI Resources
Europe				
<i>Ixodes ricinus</i>	Castor bean tick	<i>Borrelia</i> spp <i>Borrelia miyamotoi</i> TBE virus <i>Candidatus</i> Neoehrlichia mikurensis <i>Anaplasma phagocytophilum</i> <i>Francisella tularensis</i> Louping ill virus <i>Babesia divergens</i> , <i>Babesia microti</i> , other <i>Babesia</i> spp. <i>Rickettsia helvetica</i>	LD <i>Borrelia miyamotoi</i> disease TBE Neoehrlichiosis Tick-borne fever of ruminants, HGA Tularemia Louping ill of sheep and grouse Babesiosis Human SFGR	Colonies; UTMB
Asia-Pacific				
<i>Ixodes holocyclus</i>	Australian paralysis tick	<i>Rickettsia australis</i> <i>Coxiella burnetii</i>	Tick paralysis	Field specimens;
<i>Ixodes persulcatus</i>	Taiga tick	<i>Borrelia</i> spp. <i>Borrelia miyamotoi</i> TBE virus <i>Anaplasma phagocytophilum</i> , <i>Ehrlichia</i> spp.	LD <i>Borrelia miyamotoi</i> disease TBE HGA Ehrlichiosis	-
Africa				
<i>Ixodes rubicundus</i>	South African Karoo paralysis tick	No known etiological agents	Tick paralysis	Laboratory colonies; Univ. South Africa
Minor species				
<i>Ixodes affinis</i>		<i>Borrelia burgdorferi</i>	LD	-
<i>Ixodes uriae</i>	Seabird tick	<i>Borrelia garinii</i>	LD	-

<i>Ixodes cookei</i>	Powassan virus	Powassan encephalitis	-
<b><i>Ixodes</i> cell lines</b>			
Species	Name of Cell Line	Origin of Cell Line	
<i>Ixodes scapularis</i>	ISE6, ISE18, IDE8	United States	
<i>Ixodes ricinus</i>	IRE/CTVM19, IRE/CTVM20	United Kingdom	
<i>Ixodes ricinus</i>	IRE11	Germany	

HGA, Human granulocytic anaplasmosis; LD, Lyme disease; SFGR, Spotted fever group rickettsiosis; TBE, Tick-borne encephalitis; UTMB, University of Texas Medical Branch, Galveston, Texas, USA

<sup>a</sup>NIH, NIAID BEI Resources Repository: <https://www.niaid.nih.gov/research/bei-resources-repository>



Fig-1

